Acta Dermatovener (Stockholm) 61: 147–183, 1981

SHORT REPORTS

Pronounced Formation of 5-OH-Dopa at Enzymatic Oxidation of DOPA in the Presence of Ascorbic Acid

C. Hansson, H. Rorsman and E. Rosengren

Departments of Dermatology and Pharmacology, University of Lund, Lund, Sweden

Received September 3, 1980

Abstract. The recently discovered intermediate product in melanin formation, 5-OH-dopa, is formed in large amounts when dopa is oxidized by tyrosinase in the presence of ascorbic acid.

Key words: Dopa; 5-OH-dopa; Cysteinyldopa; Tyrosinase; Melanin; Ascorbic acid

5-OH-dopa is a catecholic amino acid which is formed when tyrosine or dopa is oxidized by tyrosinase (4). So far it has been detected only in relatively small quantities. In elaborating a new method for the measurement of tyrosinase activity (5), we have observed that ascorbic acid increases the production of 5-OH-dopa strikingly. Experiments which demonstrate this effect and at the same time suggest possible reaction routes for the formation of 5-OH-dopa will be described.

MATERIAL AND METHODS

Chemicals: L-dopa, L-cysteine and L(+)-ascorbic acid were of analytical grade from Merck. 5-OH-dopa was obtained from Hoffmann-La Roche. 5-S- and 2-S-cysteinyldopa were prepared according to the method of Prota et al. (6).[1] 2,5-S,S-dicysteinyldopa was prepared as previously described (1). Mushroom tyrosinase (Sigma 2230 U/mg) contained 6 ng dopa/mg but no 5-OH-dopa or cysteinyldopas. Dopa, cysteinyldopas and 5-OH-dopa were determined by HPLC as described previously (3, 4). All incubations were performed for 5 min with 1.5 mg

[1] 5-S- and 2-S-cysteinyldopa were a gift from Dr. S. Ito, Institute for Comprehensive Medical Science, Fujita-Gakuen University, Toyoake, Japan.

Table 1. *Incubation of dopa with and without cysteine and ascorbic acid with 1.5 mg tyrosinase in 1 ml 0.5 M phosphate buffer, pH 6.5 at 24.8°C*

All figures represent μ moles. Quantity of dopa was 1.0 μ mole at the start of the incubation. Time of incubation, 5 min

	2-S-cysteinyl-dopa	2,5-SS-di-cysteinyl-dopa	5-OH-dopa	Dopa	5-S-cysteinyl-dopa
10^{-3} M dopa					
	–	–	–	0.10	–
	–	–	–	0.08	–
10^{-3} M dopa 10^{-2} M ascorbic acid					
	–	–	0.11	0.86	–
	–	–	0.10	0.84	–
10^{-3} M dopa 10^{-2} M cysteine					
	0.22	0.042	–	–	0.86
	0.23	0.050	–	–	0.87
10^{-3} M dopa 10^{-2} M acorbic acid 10^{-2} M cysteine					
	0.19	0.003	0.087	0.037	0.79
	0.19	0.003	0.092	0.036	0.79

tyrosinase in 1 ml 0.5 M phosphate buffer, pH 6.5, at 24.8°C, with constant flow of 3.1 ml air/min.

The following concentrations of chemicals were used for incubations; dopa, 10^{-3} M, cysteine and ascorbic acid, 10^{-2} M. Control incubations with tyrosinase boiled for 15 min were performed.

RESULTS AND COMMENTS

The incubates containing dopa, or dopa plus cysteine did not produce detectable amounts of 5-OH-dopa (Table 1), but with ascorbic acid the quantities of 5-OH-dopa were, after 5 min, 9–11% of the dopa originally present. It should be noted, however, that 5-OH-dopa can be detected in low concentrations when incubating dopa with tyrosinase also in the absence of ascorbic acid when conditions and analytical techniques are optimized for demonstration of 5-OH-dopa (4).

In the experiment with dopa and ascorbic acid, much of the dopa consumed was recovered as 5-OH-dopa. The large quantities of dopa remaining after 5 min in this experiment are not due to the absence of dopaquinone formation but to effective reduction of the quinone formed. This is evident from the incubates containing cysteine, a strongly nucleophilic substance avidly adding to dopaquinone to give cysteinyldopa.

In our report on the appearance of 5-OH-dopa during melanogenesis from dopa we discussed the possibility of nucleophilic addition of water to dopaquinone. This type of reaction, explored by Fieser and Peters (2), has been suggested to occur upon the oxidation of catechol, by Wagreich & Nelson (7).

The formation of large amounts of 5-OH-dopa in the experiment with dopa/ascorbic acid/cysteine is firm evidence against the possibility of 5-OH-dopa production by nucleophilic addition of water, since the strongly nucleophilic SH-group of cysteine would prevent such a reaction. It seems more likely that the production of 5-OH-dopa is due to an enzymatic hydroxylation of dopa which may be enhanced by ascorbic acid. In incubations with boiled tyrosinase, dopa and ascorbic acid, dopa was quantitatively recovered and there was no detectable formation of 5-OH-dopa.

Inhibited oxidation of 5-OH-dopa may be a further explanation for the high concentrations of this substance observed in the presence of ascorbic acid.

The present finding of large quantities of 5-OH-dopa formed upon oxidation of dopa in the presence of ascorbic acid will open the way to investigations into the mechanism for 5-OH-dopa production and the role of tyrosinase. This model will also be most helpful in studying the importance of 5-OH-dopa in melanogenesis.

ACKNOWLEDGEMENTS

This investigation has been supported by grants from the Swedish Cancer Society (project 626-B81-09XC), the Walter, Ellen and Lennart Hesselman Foundation for Scientific Research, the Thure Carlsson Foundation for Scientific Research, the Edvard Welander Foundation for Scientific Research, the donative funds of the University Hospital at Lund, and the Swedish Medical Research Council (B80-14X-00056-16B).

REFERENCES

1. Agrup, G., Hansson, C., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Mass spectrometric analysis of enzymatically synthesized cysteinyldopa isomers. Communications from the Department of Anatomy, University of Lund, Sweden, No. 5, 1976.
2. Fieser, L. F. & Peters, M. A.: The potentials and the decomposition reactions of orthoquinones in acid solution. J Am Chem Soc *53:* 793, 1931.
3. Hansson, C., Agrup, G., Rosman, H., Rosengren, A.-M. & Rosengren, E.: Analysis of cysteinyldopas, dopa, dopamine, noradrenaline and adrenaline in serum and urine using high-performance liquid chromatography and electrochemical detection. J Chromatogr *162:* 7, 1979.
4. Hansson, C., Rorsman, H. & Rosengren, E.: 5-Hydroxydopa, a new compound in the Raper-Mason scheme of melanogenesis. Acta Dermatovener (Stockholm) *61:* 281, 1980.
5. — A method for measurement of tyrosinase activity. Acta Dermatovener (Stockholm), to be published.
6. Prota, G., Scherillo, G. & Nicolaus, R. A.: Struttura e biogenesi delle feomelanine. Nota IV. Sintesi e proprietà della 5-S-cisteinildopa. Gazz Chim Ital *98:* 495, 1968.
7. Wagreich, H. & Nelson, J. M.: On the mechanism of the catechol-tyrosinase reaction. J Am Chem Soc *60:* 1545, 1938.